

Figure S1. Design of the time-course experiment. (a) Using a hydroponic system, *A. thaliana* seedlings of two different genotypes (WT and *nia1/nia2* double mutant) were grown for 30 days (days after germination; dag) in MS media supplemented with either 0.2 mM (N deprivation condition; nutritional stress) or 4 mM (optimal N concentration). The sampling points selected to analyze phenotypal traits and perform the metabolomic studies were 15 and 30 dag. The rosettes (shoots) and roots were harvested separately. Six plants were germinated in each hydroponic chamber, which was ordered as shown in (b) panel. Three biological replicates were made for each treatment.

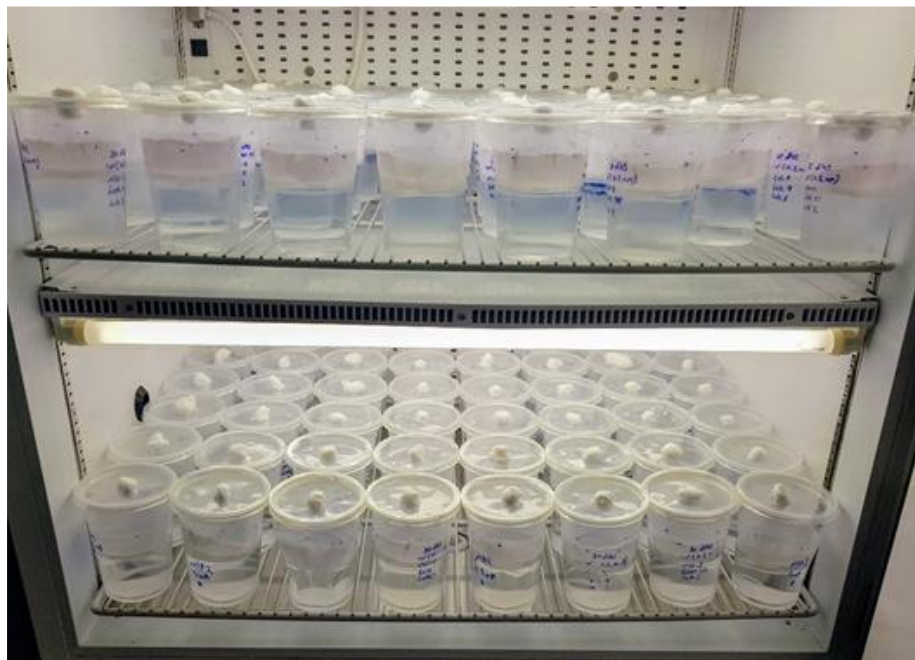


Figure S2. Seedling growth under aseptic and controlled conditions. The hydroponic chambers in which *A. thaliana* seedlings were sown were accommodated into a growth chamber with fluorescent light, constant temperature (22 °C) and using a photoperiod of 16 h light/8 h dark (see methods for details).

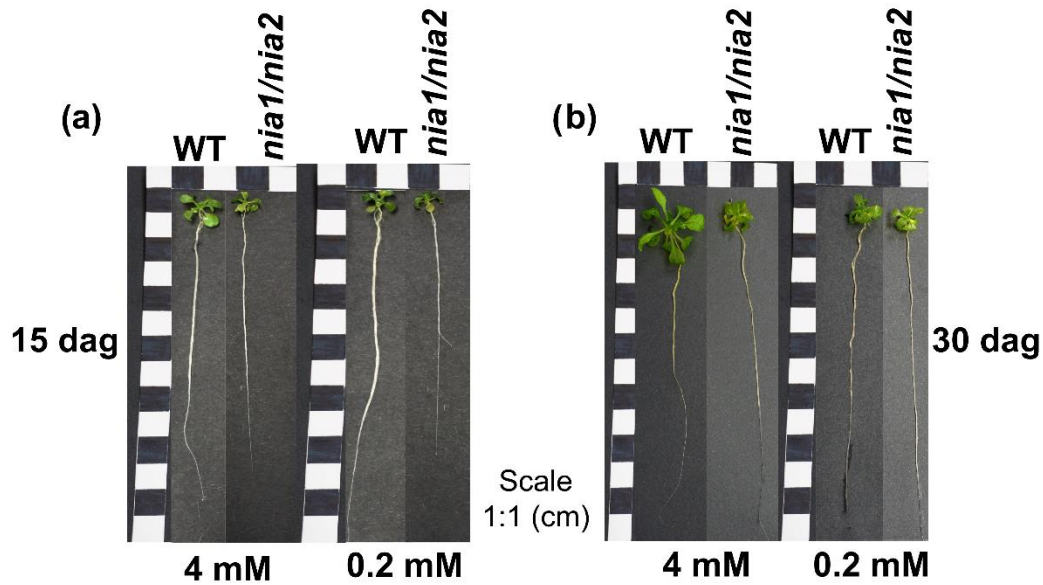


Figure S3. Phenotypic traits of *Arabidopsis thaliana* seedlings. Seedlings were harvested from the hydroponic systems. The rosettes (shoots) and roots from both genotypes (WT and *nia1/nia2* double) are shown at (a) 15 dag and (b) 30 dag.

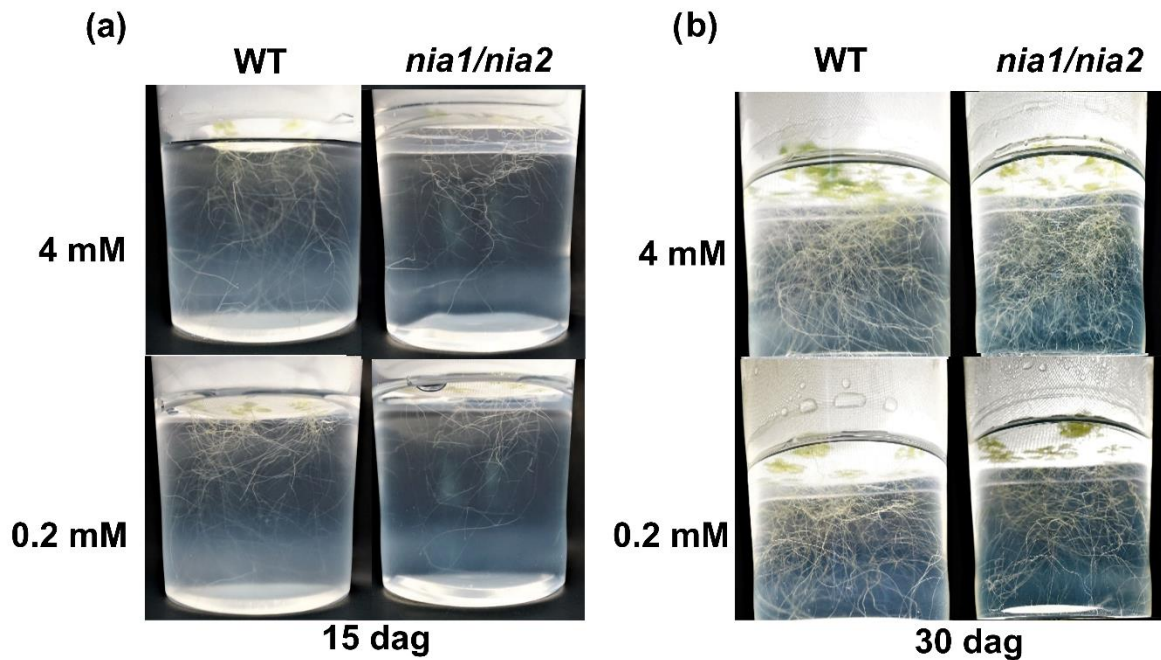


Figure S4. View of roots growth into the hydroponic chambers. Elongation and growth of roots from the WT and the *nia1/nia2* double mutant genotypes at (a) 15 dag and (b) 30 dag. Roots' growth at both N availability conditions (4 mM and 0.2 mM) are shown.

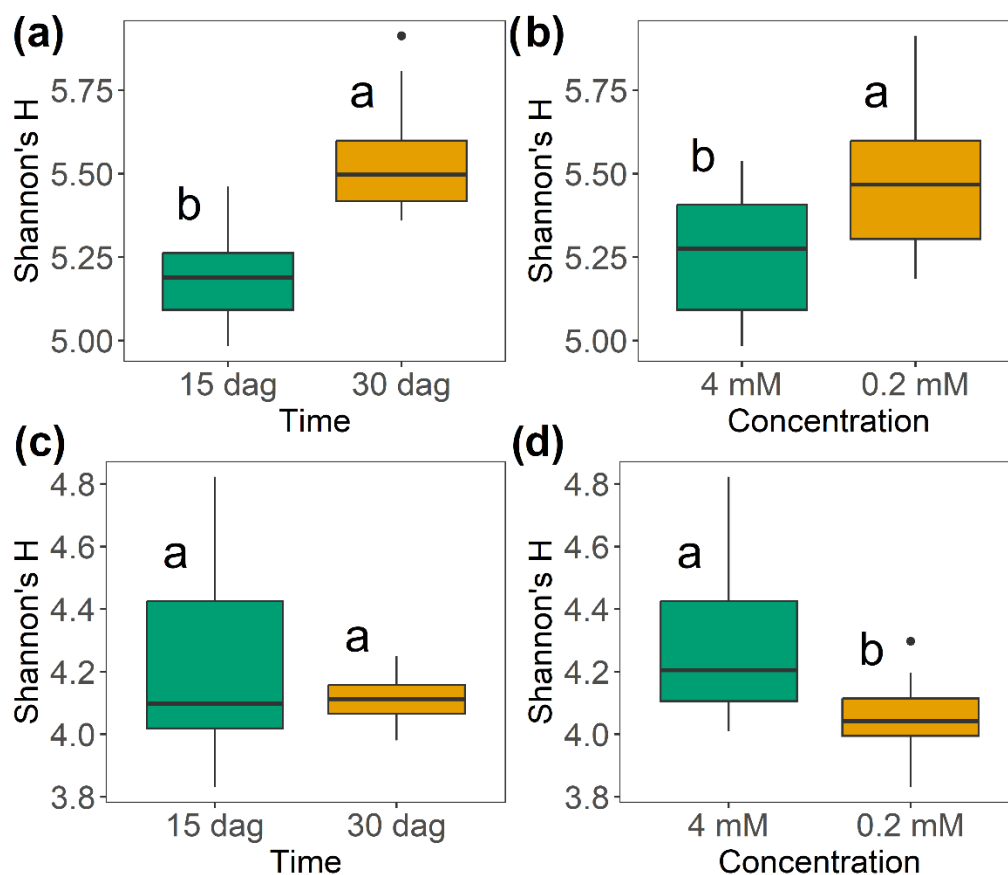


Figure S6. Analysis of variance (one-way ANOVA) of the *A. thaliana* metabolome obtained from each ESI mode. (a) and (b) show, for the ESI⁺ mode, the differences between the sampling points (15 and 30 dag) and N concentrations (4 and 0.2 mM), respectively. Same, but from ESI⁻ mode is shown in (c) and (d) panels. The boxplot graphs represent the mean of the data, the dispersion, and the interquartile range delimited by quartiles 1 (Q1) and 3 (Q3). The small letters above the boxplots represent Tukey's *post-hoc* test clustering.

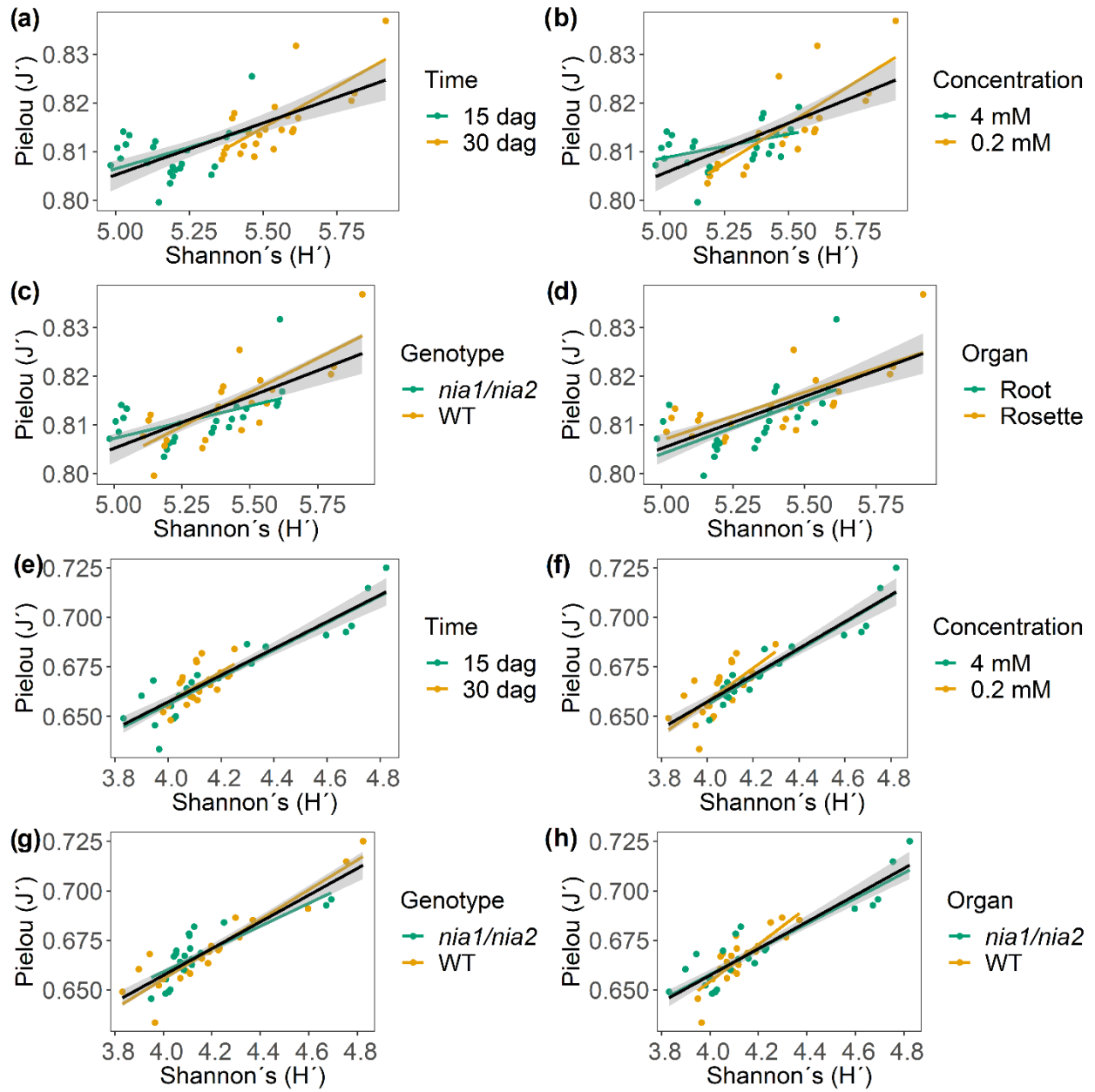


Figure S7. Pearson correlation and covariance between the Pielou (J') and diversity. ESI modes were analyzed independently. Panels (a), (b), (c), and (d) correspond to ESI+ mode, while (e), (f), (g), and (h) panels represent ESI- mode. In both ESI modes, obtaining positive correlative and covariance values stands out. The lines in mustard and green color correspond to the linear model of the correlation for each variable. The black line corresponds to the optimal theoretical linear distribution model, and the shadow in grey to its confidence intervals (95%).

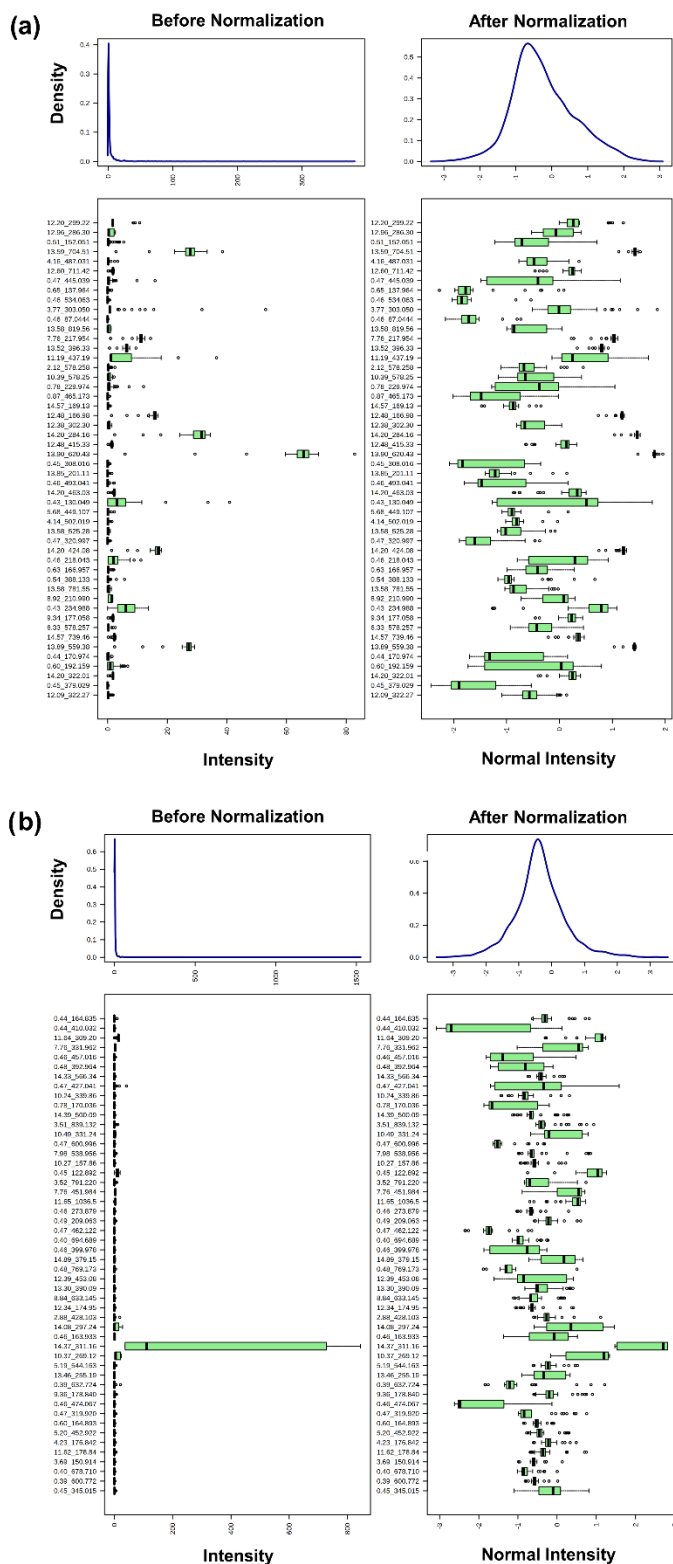


Figure S8. Spectral libraries normalization. For both ionization modes, (a) ESI⁺ and (b) ESI⁻, box plots show the range of intensities detected from each m/z_{rt} . The intensities distribution is shown for both raw data (left), and once they were normalized by quantile and log transformation (right). A maximum of 50 m/z_{rt} was shown due to space limitations in the graph. Besides, kernel density plots are also shown at the top of box plots. Notice that once normalized, the intensities range shows a normal distribution.

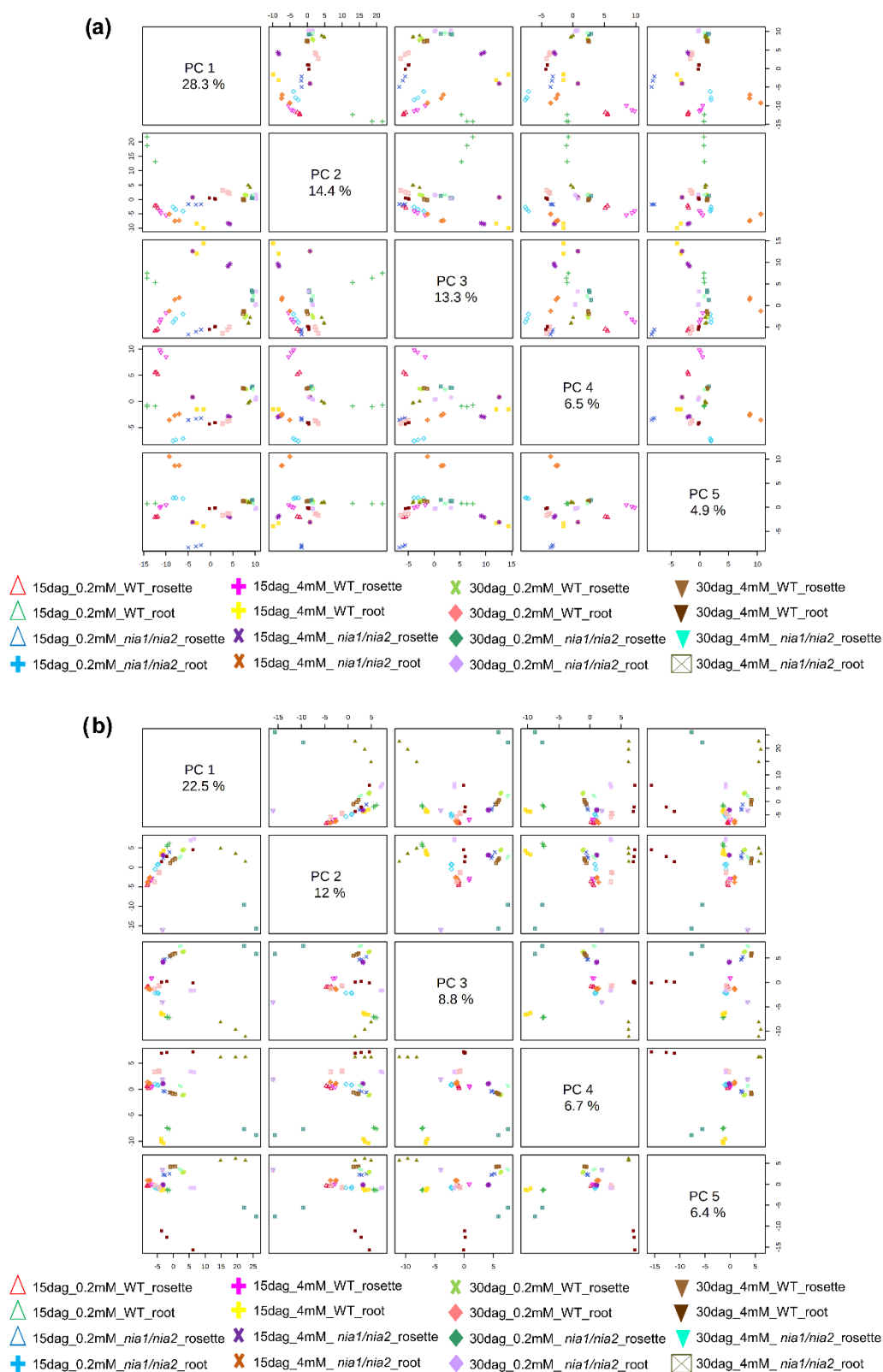


Figure S9. Principal component analysis (PCA). The explanatory percentage of variance values is shown for (a) the ESI⁺ mode and (b) the ESI⁻ mode. The first principal component (PC1) has the highest variance, followed by the second principal component (PC2).

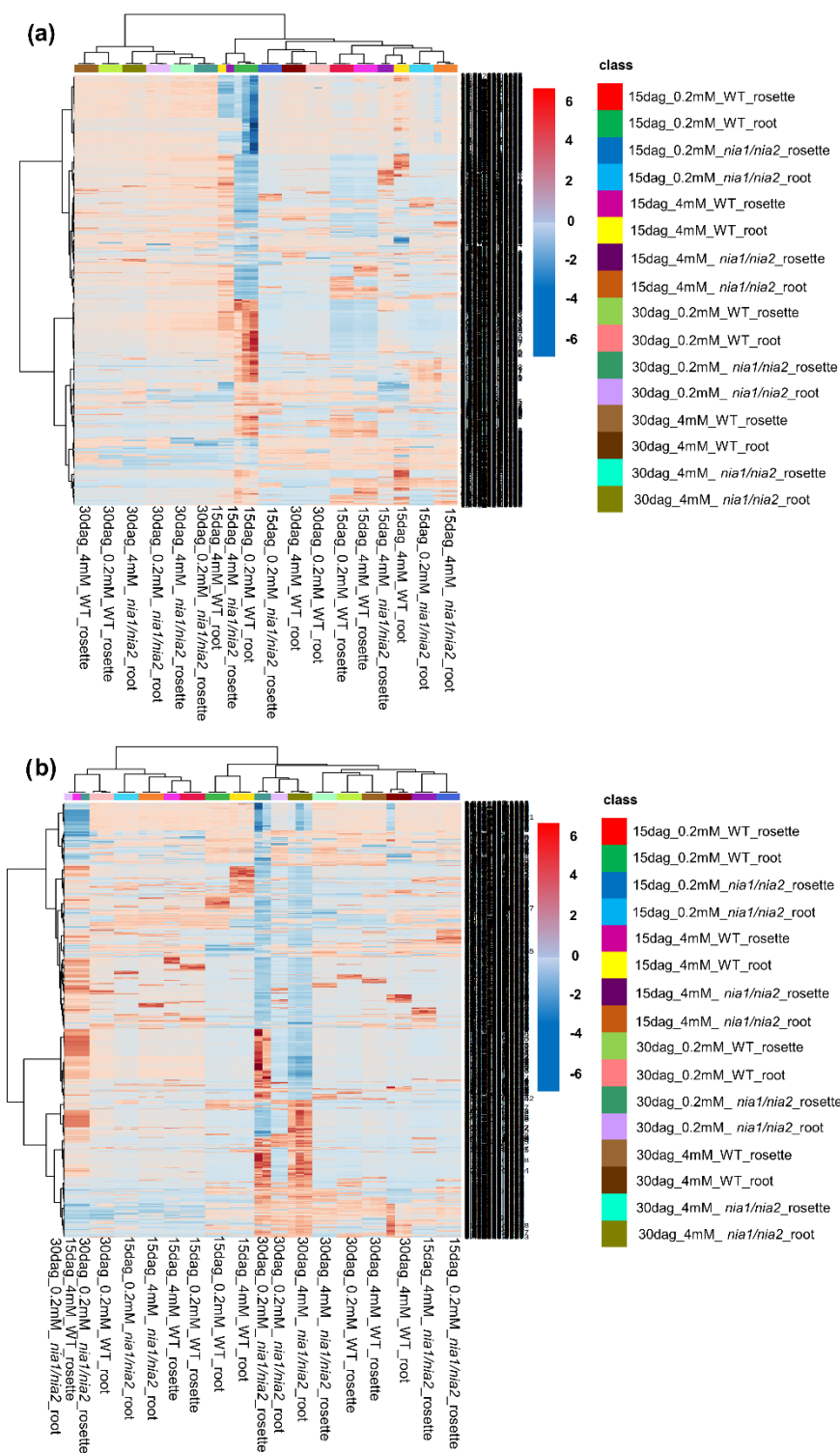


Figure S11. Heatmaps of the metabolomics dataset. The colors represent changes of each m/z_{rt} relative to the mean control level. Samples (*x*-axis) and m/z_{rt} (*y*-axis) are separated using hierarchical clustering (Ward's algorithm), with the dendrogram being scaled to represent the distance between each branch (distance measure: Pearson's correlation). **(a)** Show those genes identified in mass spectral libraries obtained in ESI⁺ mode, while **(b)** show the same for ESI⁻ mode.

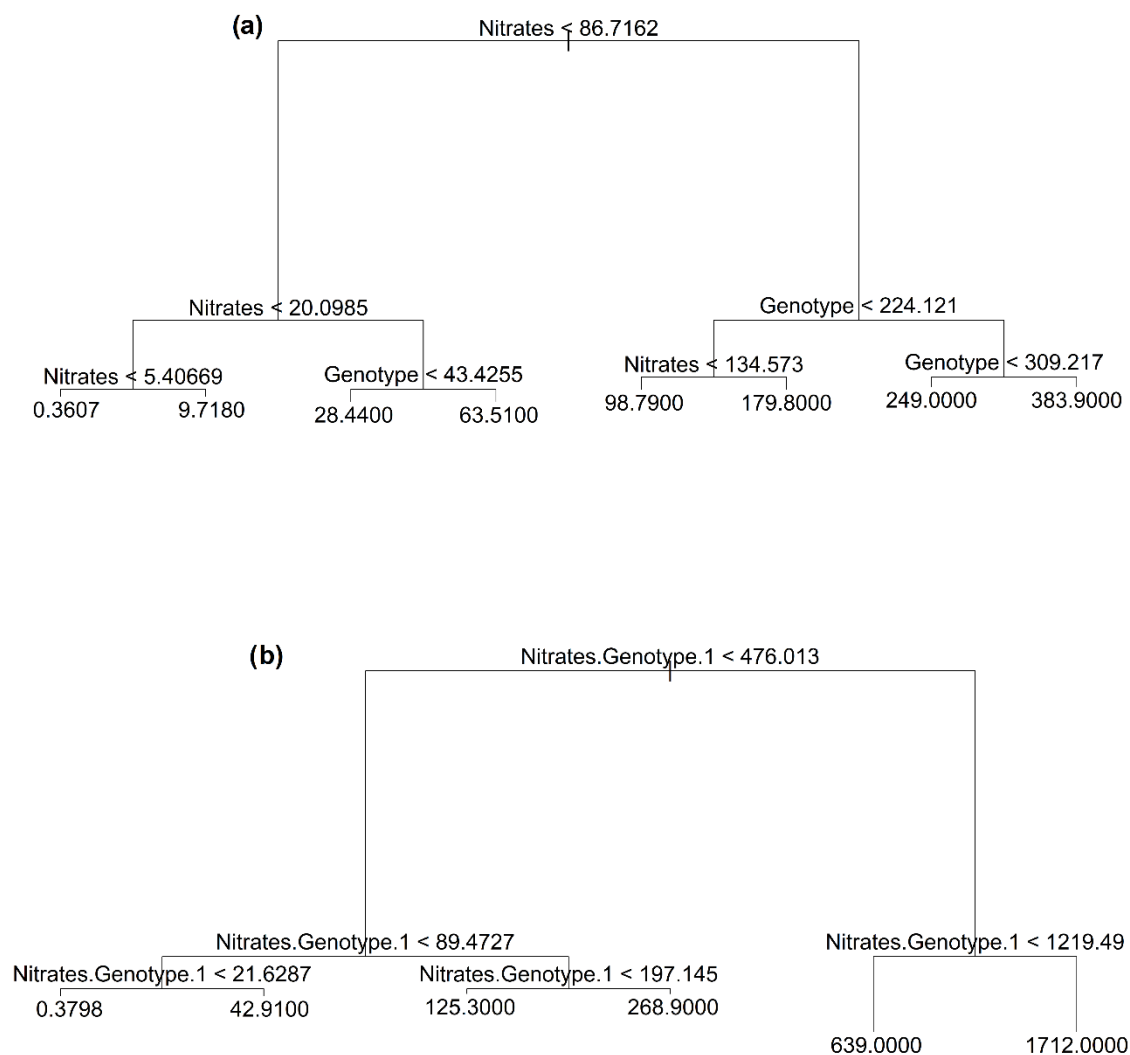


Figure S12. Decision trees (regression trees) and the variable importance measures (genotypes and N concentrations) from sampling points. **(a)** illustrates a regression tree from the dataset obtained in ESI⁺ mode. On the first split, the decision tree separates nitrates concentrations from genotypes. The tree is pruned to the size that has the lowest cross-validation error. At each terminal node, the average relative abundance of m/z_{rt} allocated to the node is shown. **(b)** panel show the same but from the dataset obtained in ESI⁻ mode.

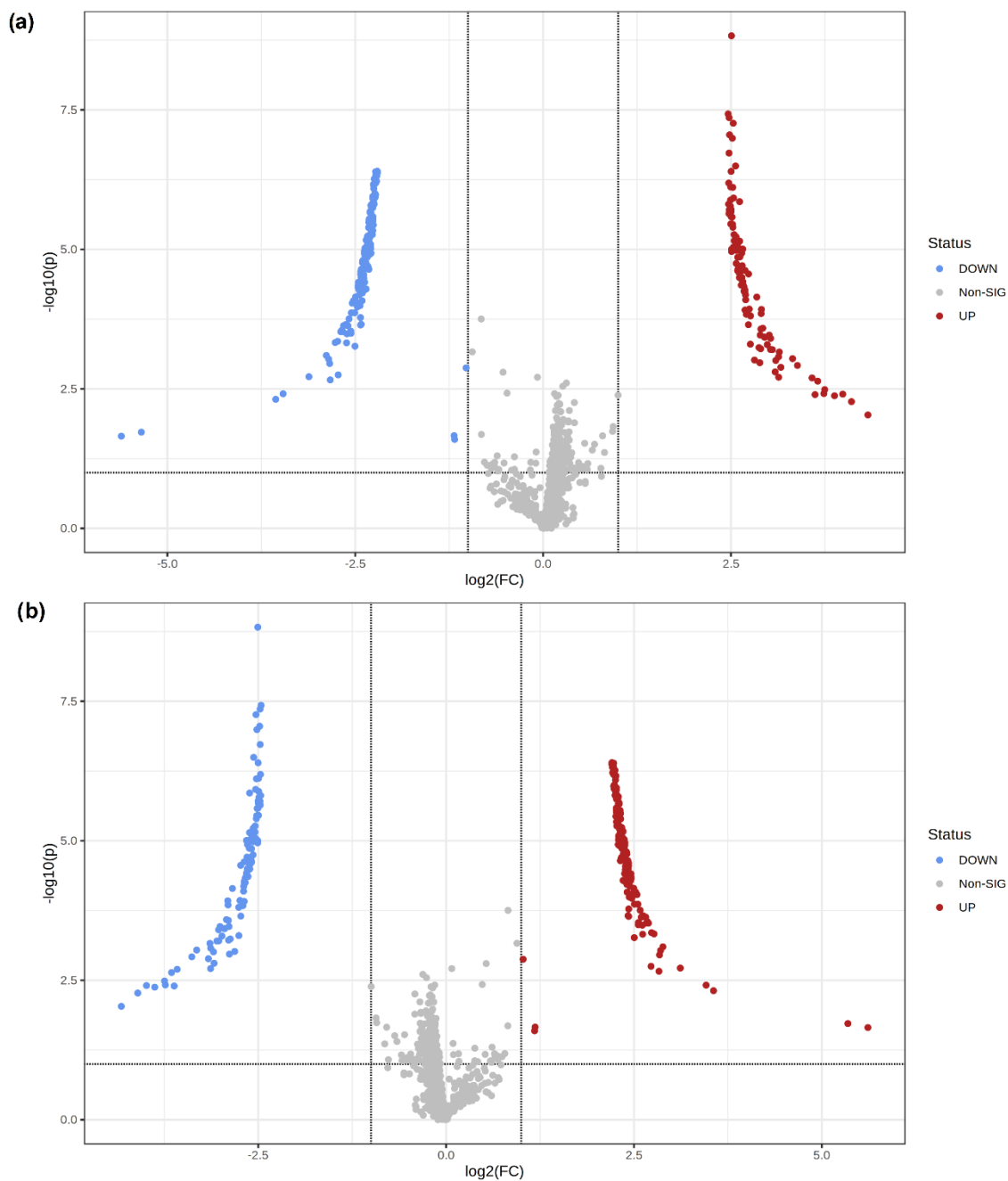


Figure S13. Volcano plots showing the differentially synthesized/accumulated m/z values. According to pairwise comparisons performed (Table S9), differentially synthesized/accumulated m/z values were identified. (a) illustrates, as an example, the differentially synthesized/accumulated m/z values identified in ESI⁺ mode from the contrast 15dag_4mM_WT_rosette versus 15dag_0.2mM_WT_rosette. (b) Same contrast but from those differentially synthesized/accumulated m/z values identified in ESI⁻ mode. Datasets were filtered to remove m/z values with low relative abundance (dotted line from -1 to 1 on the x -axis), and a significance cut-off (p -value ≤ 0.01) was applied (dotted line on the y -axis).

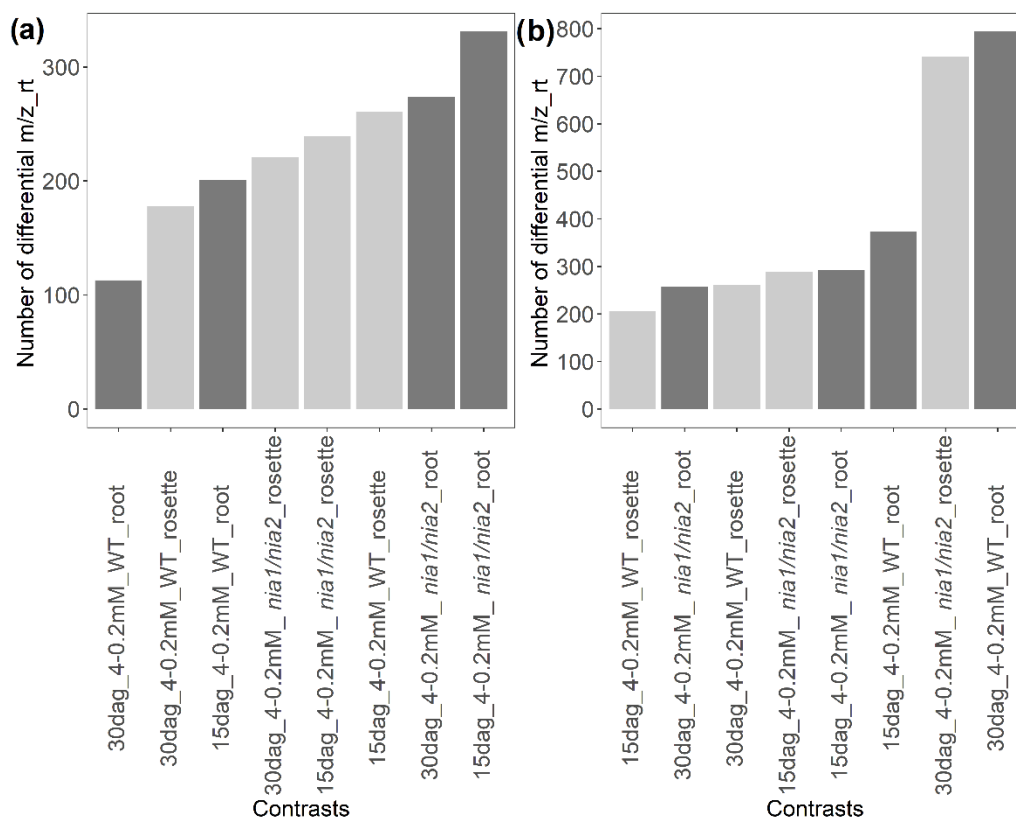
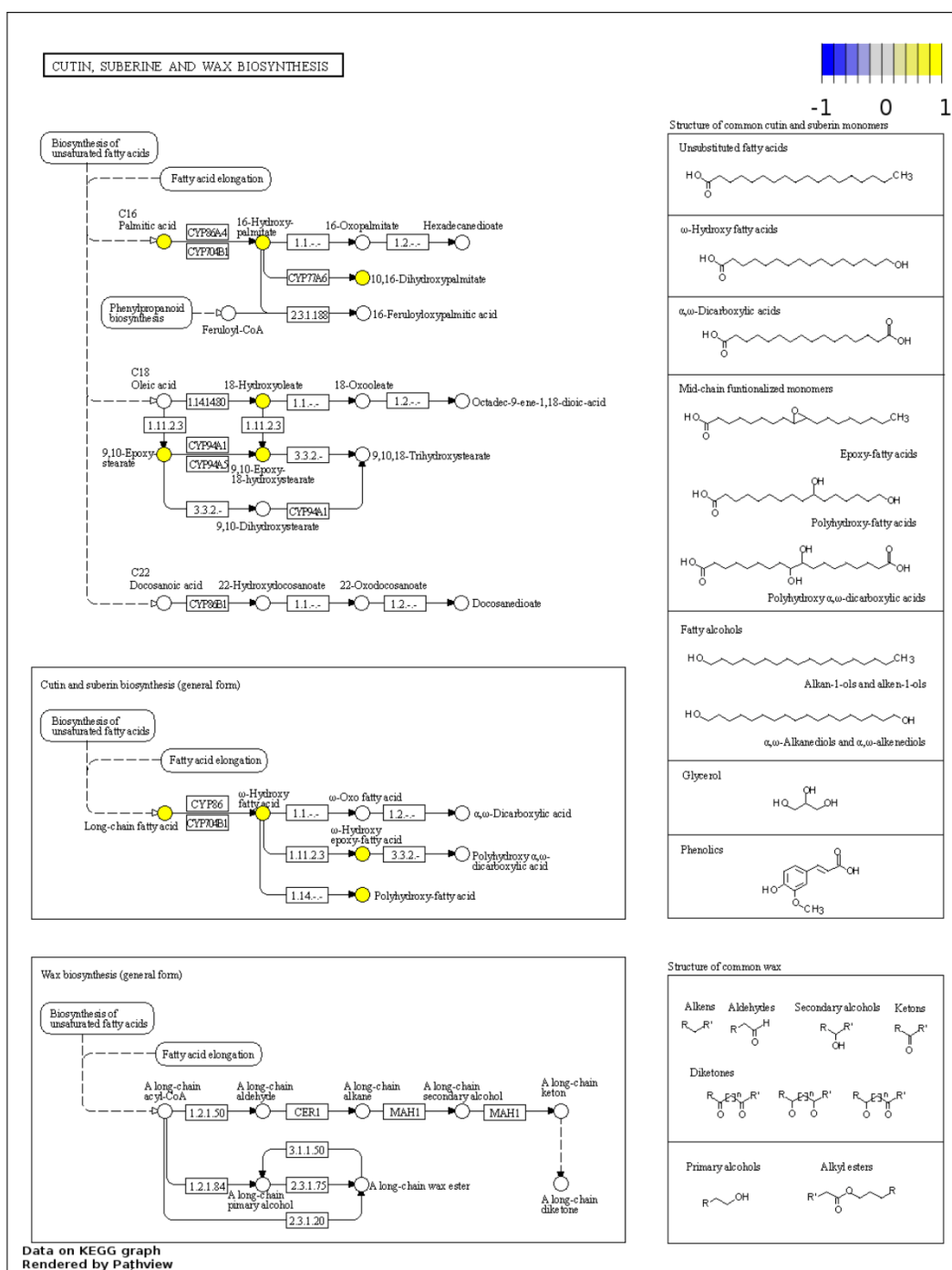
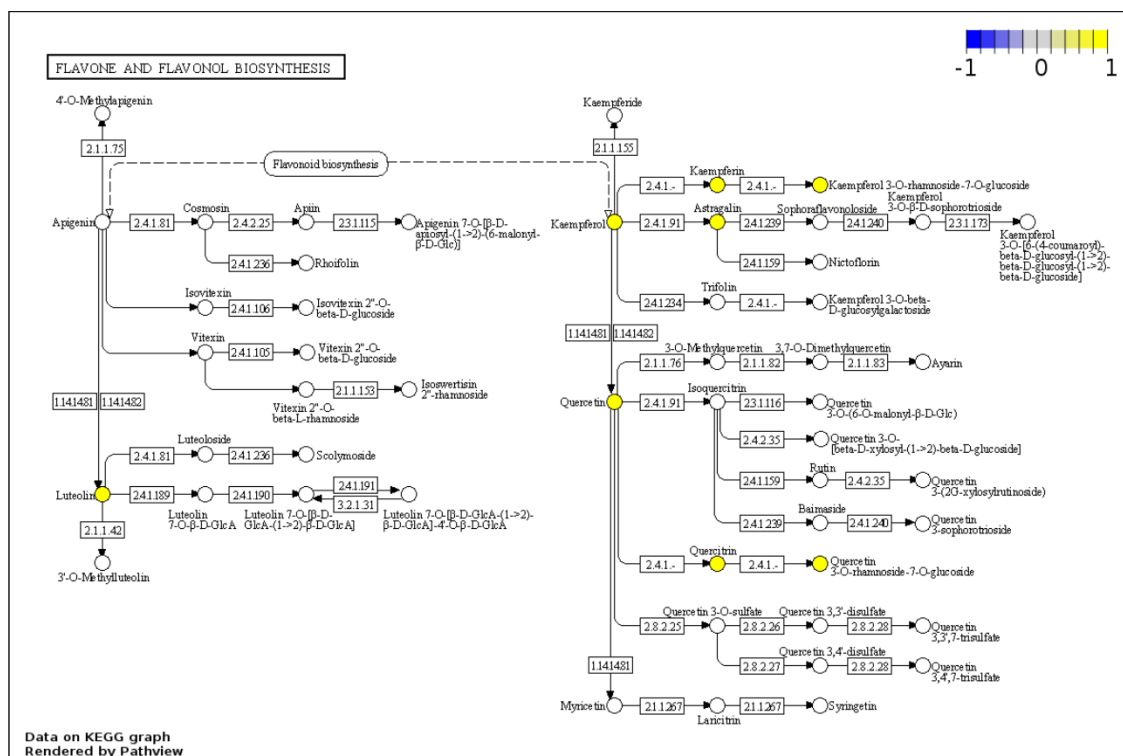


Figure S14. Bar plots summarizing the total of the differentially synthesized/accumulated m/z_{rt} . According to each pairwise comparison performed (x -axis, see details in Table S9), the total of differentially synthesized/accumulated m/z_{rt} (y -axis) is shown. The number of differentially synthesized/accumulated m/z_{rt} is ordered from smallest to largest and is shown independently for both ESI modes. **(a)** ESI⁺ and **(b)** ESI⁻.

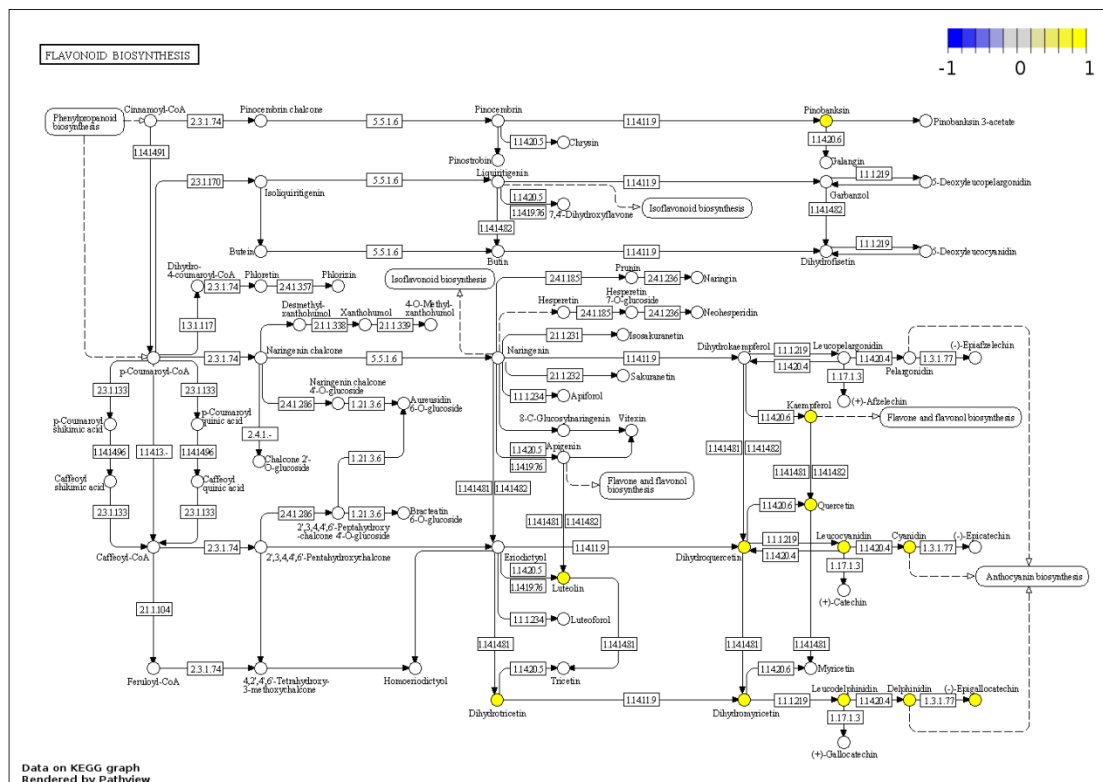
(a)



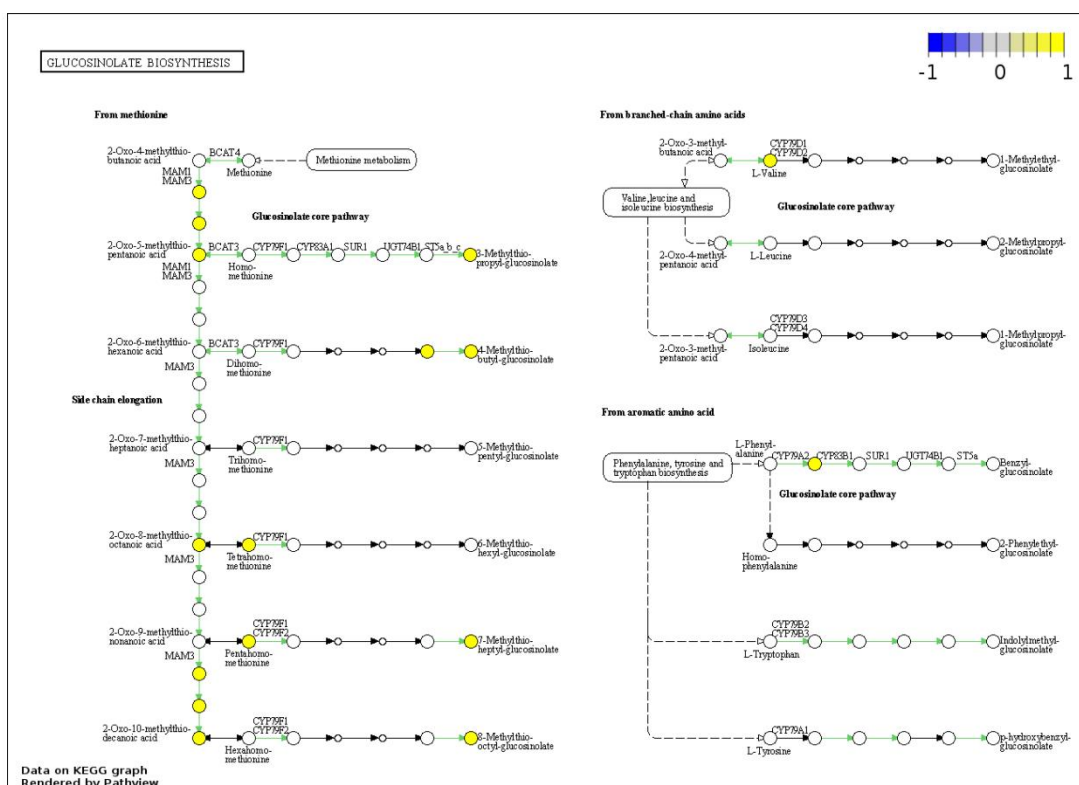
(b)



(c)



(d)



(e)

